

Awad-George 4.1-7  
Appl. No. 10/679,714  
Amendment dated: February 13, 2008  
Reply to Final Office Action dated October 18, 2007

#### **REMARKS**

Claims 1, 2, 4, 6-14 and 16-19 are pending. Claims 3, 5 and 15 were previously cancelled. No claims are allowed.

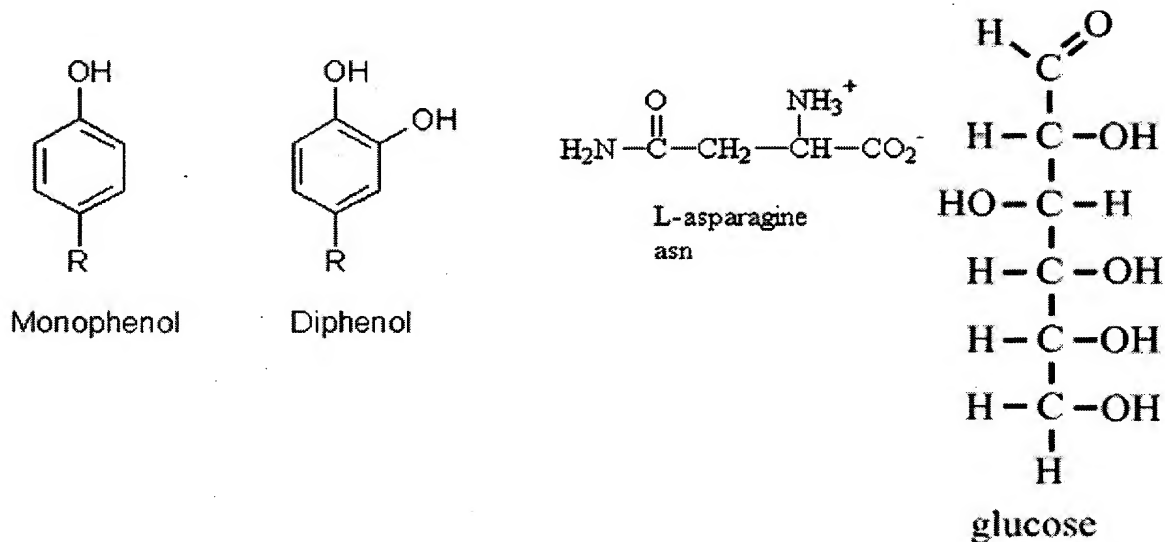
The Reply to the Final Office Action dated October 18, 2007, was entered. The claims have been further amended to clearly reflect the addition of the raw, uncooked food into a fermenter. An outlet strainer for straining the liquid from the fermented food in the fermenter is provided. The raw food is then washed by introducing water into the fermenter to remove residues on the food. Claim 1 has been amended to call for "free asparagine" in the food as seen in paragraph [0006] and in Table 1 of the specification.

#### **Office Action:**

The Office Action stated that: "Hilton states on column 2, lines 61-62, that" it is preferred to blanch the potato solids to reduce their tendency to brown enzymatically." This teaches that blanching has been known in the art to reduce browning in potato pieces."

**Response:**

Foods turn brown through an enzymatic or non-enzymatic process (Maillard reaction). Enzymatic browning is one of the most important color reactions that affects fruits, vegetables and seafoods. It is catalyzed by the enzyme polyphenol oxidase. Polyphenol oxidase catalyses the initial step in the polymerization of phenolics to produce quinones, which undergo further polymerization to yield dark, insoluble polymers referred to as melanins. Polyphenol oxidase catalyses two basic reactions: hydroxylation to the o-position adjacent to an existing hydroxyl group of the phenolic substrate (monophenol oxidase activity), and oxidation of diphenol to o-benzoquinones (diphenol oxidase activity) [Reference 1]. Enzymatic browning has nothing to do with acrylamide formation. It is a completely different reaction than the acrylamide reaction which takes place when starch-based foods such as potatoes are fried or baked at temperatures higher than 120°C. The precursors of acrylamide are asparagines and reducing sugars (glucose etc.) and these are not substrates for the enzyme polyphenol oxidase.



Enzymatic browning is usually controlled as suggested by Hilton et al. by blanching (column 2, lines 61-62) or with chemicals such as sulfite and sulfur dioxide as also suggested by Hilton et al.:" the potatoes may be treated with chemical agents such as sulfite or sulfur dioxide to reduce discoloration (column 3 lines 4-6)" to destroy the enzyme polyphenol oxidase to preserve color (inhibition or reduction of browning) in vegetables such as potatoes. Inhibition of enzymatic browning is completely different than inhibition of acrylamide formation. The 2 reactions are not even remotely related.

**Office Action:**

"It is noted that the prior art to Hilton, although not explicitly reciting the reduction of acrylamide, achieves this result since Hilton et al. teach that the prefermented potatoes have a low tendency to brown during frying. The formation of acrylamide (and browning) is a direct result of the reaction of asparagine (found in potatoes) with reducing sugars. Therefore, since Hilton et al. lowers the levels of the reducing sugars, one component of the Maillard reaction equation is minimized, thus also lowering the end result (i.e. acrylamide levels and browning)."

**Response:**

Reducing acrylamide precursors (asparagine or reducing sugars) in starch based foods whether by fermentation or other means reduces the formation of acrylamide in these foods after frying. In fact, in the claimed invention when the amount of reducing sugars was reduced to less than 0.1% by fermentation with active dry yeast (*Saccharomyces cerevisiae*) at pH 7 (Table 8 page 16 specification), the observed acrylamide reduction was

20%. However, when the amount of reducing sugars was also reduced to less than 0.1% by fermentation with active dry yeast (*Saccharomyces cerevisiae*), but the pH was adjusted to pH 4 prior to the onset of the fermentation, the acrylamide reduction observed was 74%. By changing the pH from pH 7 to pH 4 at the same reducing sugar levels (<0.1%), there was 54% increase in the reduction of acrylamide.

**TABLE 8** (Specification page 16)

**Effect of pH using active dry yeast (*Saccharomyces cerevisiae*)**

Yeast (g)	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction (%)	Mono & di-saccharides (%)
2.5	4	30	0.5	100	2.5	74	<0.1
2.5	5	30	0.5	100	2.5	60	<0.1
2.5	6	30	0.5	100	2.5	23	<0.1
2.5	7	30	0.5	100	2.5	20	<0.1
2.5	8	30	0.5	100	2.5	25	<0.1

The same trend was observed with lactic acid bacteria (Table 9, specification page 16). When the amount of reducing sugars was reduced to less than 0.1% by fermentation with lactic acid bacteria at pH 7, the observed acrylamide reduction was 45%. However, when the amount of reducing sugars was also reduced to less than 0.1% by fermentation with lactic acid bacteria, but the

pH was adjusted to pH 4 prior to the onset of the fermentation, the acrylamide reduction observed was 81%. By changing the pH from pH 7 to pH 4 at the same reducing sugar levels (<0.1%), there was 36% increase in the reduction of acrylamide.

**TABLE 9 (Specification page 16)**

**Effect of pH using bacterial cells (*Streptococcus thermophilus*)**

Bacterial (count)	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction (%)	Mono & di-saccharides (%)
5x10 <sup>9</sup>	4	30	0.5	100	2.5	81	<0.1
5x10 <sup>9</sup>	5	30	0.5	100	2.5	72	<0.1
5x10 <sup>9</sup>	6	30	0.5	100	2.5	64	<0.1
5x10 <sup>9</sup>	7	30	0.5	100	2.5	45	<0.1
5x10 <sup>9</sup>	8	30	0.5	100	2.5	31	<0.1

Surprisingly, at lower pH the acrylamide reduction was much higher than the ones observed at the optimal pH (6-7) for microbial growth, even though at both pH's the amount of reducing sugars (acrylamide precursors) was the same <0.1%. The higher acrylamide reduction obtained at pH 4 indicates that a low pH has an effect on the acrylamide reduction independent of the amount of reducing sugars present. Since, in this case, the amount of reducing sugar was the same <0.1%, other mechanisms

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different than Maillard reaction probably operates to reduce acrylamide formation after frying. Hilton et al. did not use a pH adjusting step whether prior, during or after their yeast fermentation process. In contrast, in the claimed invention, the Applicant intercalated a pH adjusting step prior to the onset of the fermentation to increase the reduction of acrylamide.

In view of the prior art cited, it would not have been obvious to one having ordinary skill in the art at the time of the invention to have employed a pH pre-adjusting step in conjunction with removal of acrylamide precursors by microbial fermentation in the processes of the cited art and motivation for doing so is simply not suggested. Applicant's extensive analysis of the variables involved and discussion with respect to the pertinent prior art constitute a solid ground for the patentability of the instant invention.

**Office Action:**

"On page 32, Applicant states that the Baldwin reference is directed to an enzymatic process and teaches against the use of fermentation by lactic acid or yeast.. The Examiner recognizes that obviousness can only be

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established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988) and *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In this case, Baldwin teaches that it has been known in the art to use lactic acid bacteria for fermentation which reduces browning."

**Response:**

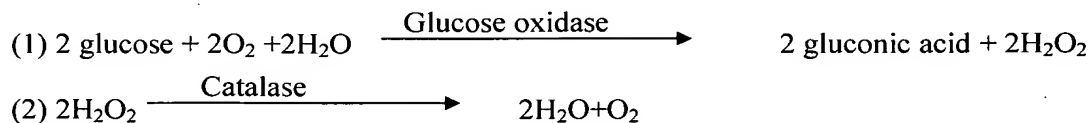
In fact, not just Baldwin taught that it has been known in the art to use lactic acid bacteria for fermentation. The Applicant reported: "The use of acid producing bacterial cultures for food fermentation is well known" (specification page 2, paragraph [0003], lines 4-5) and the Applicant also reported: "The use of yeast fermentations is also well known: (specification page 2, paragraph [0004], lines 1-2). However, the prior art reported by Baldwin in his introduction about the use of lactic acid bacteria to reduce browning did not remotely suggest the intercalation of a pH adjusting step



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prior to the onset of the fermentation as claimed by the Applicant. In fact, it defies logic to adjust the pH to 4, because it precipitates most of the egg white proteins, especially those that are responsible for the whipping characteristics of the final product, which precipitates at pH 6, as reported by Baldwin. In fact, Baldwin stated (column 1, line 70 to column 2, line 18): "However, when the hydrogen ion concentration of the eggs undergoing fermentation is allowed to fall as low as pH 6.0, a heavy scum forms on the surface of the fermenting mixture. This scum is due to the precipitation of the mucin and mucoid fractions normally present in egg whites. This scum must be removed from the surface of the fermentation vat and may represent a loss of from 10 to 15 percent of the total solids. Inasmuch as the mucin and the mucoid fractions begin to separate from egg whites when the hydrogen ion concentration is lowered to a value below pH 6.3, substantially all of this material is lost to the final product. The mucin and mucoid fractions are very desirable in dried egg whites not only because of their nutritive value, but because they increase the whipping characteristics of the final product. Mucin or mucoid fractions which have once been

precipitated in the lactic acid fermentation in the form of scum can not effectively be reconstituted into the product since these fractions have thereby become denatured and have lost many of their desirable characteristics." Baldwin solved this problem by using the enzyme glucose oxidase in the presence of oxygen to convert glucose to gluconic acid, and decomposition of hydrogen peroxide by the catalase enzyme in accordance with the following reactions (column 3, lines 19-50):



Therefore, the prior art reported by Baldwin about the use of lactic acid bacteria to reduce browning in egg whites and the claimed invention are not related and have completely different scopes. The materials being treated, process mechanics and products are completely different and require different treatments and apparatus. The claimed invention is not capable of performing the intended use of the prior art reported by Baldwin because the pH adjusting step (pH 4) intercalated by the

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Applicant before the onset of the fermentation would lead to the precipitation of the egg white protein.

The combination of references could not possibly produce the claimed invention. Reconsideration is requested.

It is now believed that Claims 1, 2, 4, 6-14 and 16-19 are in condition for allowance. Notice of Allowance is requested.

Respectfully,



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Statement with PTO Form 1449